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# Absorption Dynamics of Organic Chemical Transport across Trout Gills as Related to Octanol–Water Partition Coefficient

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Absorption Dynamics of Organic Chemical Transport across Trout Gills as Related to Octanol–Water Partition Coefficient. McKim, J., Schmieder, P., and Veith, G. (1985). *Toxicol. Appl. Pharmacol.* 77, 1–10. An *in vivo* fish preparation was used that allowed a direct measure of the transport rates of 14 different organic chemicals across the gills of rainbow trout (*Salmo gairdneri*). The chemicals, all $^{14}C$ labeled, were selected from five classes, encompassing a range of octanol–water partition coefficient (log *P*) values, from 0.23 (ethyl formate) to 7.5 (mirex). The uptake efficiency (extraction efficiency) of each chemical was determined by monitoring the inspired and expired water of trout exposed to each chemical over an exposure period of 1 to 6 hr. The mean gill extraction efficiency for all chemicals tested varied from a low of 7% to a high of 60%, extracted in a single pass of the chemical across the gills. The extraction efficiency of chemicals with log *P* or 1 or less were low and showed no relationship to log *P*. These low extraction efficiencies seen at log *P* of 1 and below with molecular weights below 100 were indicative of aqueous pore transport. The mean extraction efficiency for chemicals with log *P* values of 1 to 3 seemed to vary directly with log *P*, to a maximum of slightly greater than 60%, suggesting that uptake was controlled by the lipid membrane. The mean extraction efficiency for chemicals with log *P* of 3 to 6 was independent of log *P* and remained at 60%, which suggested that gill uptake was controlled by aqueous diffusion rates rather than gill membrane permeability. The mean extraction efficiency with mirex (log *P* = 7.5) decreased to 20%. This behavior was consistent with previous physical models which predict that high log *P* and melting point (low water solubility) may substantially reduce the rate of accumulation in fish. These studies support the passive diffusion model for the uptake of organics at the gill–water interface. © 1985 Academic Press, Inc.

In fish toxicology the major route of entry of toxicants dissolved in water is directly across the blood–water interface at the gills. Since few active processes are known to move xenobiotics across biological membranes, they are assumed to be transported by simple, passive diffusion (LeFevre, 1972; Hunn and Allen, 1974). The physical–chemical and biological mechanisms which influence the transport of chemicals across biological membranes include; charge on the molecule, molecular weight, octanol–water partition coefficient, molecular volume, concentration in the water, surface area, and the overall metabolic rate (Spacie and Hamelink, 1982). During the last 20 years, considerable progress on understanding the mechanisms controlling membrane transport has been made by pharmacologists and drug design experts. For example, Flynn and Yalkowsky (1972) successfully demonstrated the mechanisms operating in the diffusion of nonionic organics across a physical membrane composed of dimethylpolysiloxane and bounded by two aqueous compartments. They showed membrane control, involving the octanol–water

partition coefficient, and aqueous diffusion layer control, concerning the chemical's ability to move across the aqueous boundary layers adjacent to the membrane, were responsible for transport rates. Yalkowsky *et al.* (1974) provided the first indirect biological evidence for the validity of this physical model through the relationship of biological activity (fish turnover time) and alkyl chain length of an anesthetic alkyl *p*-aminobenzoate. The initial physical model was later expanded by Yalkowsky and Morozowich (1980) to include the contribution of aqueous pores to the transport of low-molecular-weight highly polar organics.

To understand the dynamics of chemical uptake and distribution in fish we have investigated the major physical–chemical and biological mechanisms controlling absorption of organic chemicals from the respiratory water at the gill membrane. Previous work provided us with an *in vivo* fish model capable of monitoring the direct uptake (extraction efficiency) of organic chemicals across the gills (McKim and Goeden, 1982; McKim and Heath, 1983). This *in vivo* fish model closely mimicked the *in vitro* physical membrane model used by Flynn and Yalkowsky (1972) for determining passive membrane transport.

The present effort was directed at determining the influence of the octanol–water partition coefficient (log *P*) on the transport of 14 organic chemicals from five chemical classes across the gill membrane of rainbow trout. In addition, it allowed a comparison between transport rates obtained with our *in vivo* membrane model and previous results obtained with the *in vitro* physical membrane model of Flynn and Yalkowsky (1972).

## METHODS

*Animals.* The rainbow trout (*Salmo gairdneri*) used in this study weighed 660 to 840 g. They were maintained on Glencoe Mills[1] trout feed for up to 24 hr before experimentation. The fish were on a 12-hr photoperiod (incandescent) at an intensity of 11.0 lx at the air–water interface. All of the trout were acclimated to a temperature of 11 to 12°C for several months prior to experimental use.

*Metabolism chamber and surgical procedures.* The fish were subjected to a water exposure of selected organic chemicals (Table 1) in a Plexiglass respirometer–metabolism chamber, described in detail by McKim and Goeden (1982). Each fish was anesthetized with 100 mg/liter of MS222 (tricaine methanesulfonate, Finquel)[2] and immobilized by spinal transection; each was reanesthetized 48 hr later, and a latex rubber oral membrane was sutured around the mouth to separate inspired and expired water. The fish was then removed from the surgical table and placed into a respirometer–metabolism chamber. Exposure to a radiolabeled chemical began 24 hr later.

*Sample collection.* During each 2- to 4-day experiment, 7 to 12 measurements were made on each fish for ventilation volume, ventilation rate, and oxygen uptake efficiency as described by McKim and Goeden (1982). Prior to dosing, three measurements of each respiratory parameter were also made on each fish to provide control values.

Chemicals were individually introduced into the respirometer–metabolism chambers at water concentrations 10 to 100 times lower than reported 96-hr LC50 values and lower than all published solubility values (Table 1). The duration of exposure to each chemical was 2 to 6 hr during which the inspired and expired water of each fish was monitored three to six times to determine the amount of each chemical removed by a fish after flowing once across its gills (extraction efficiency). At the end of each 3- to 6-hr period, chemical dosing was stopped and the chambers were flushed with control water for 1 hr, followed by initiation of dosing with the next chemical to be tested from Table 1.

Four separate experiments were conducted utilizing two fish per experiment (except for *p*-cresol where only one fish was exposed) in individual respirometer–metabolism chambers. The first two experiments involved individual exposure to each of the seven chemicals in Table 1 (tetrachlorobiphenyl (TCB), pentachlorophenol (PCP), decanol, dodecanol, *n*-butanol, chlorobenzene, and *p*-cresol), followed by two more experiments involving individual exposure to each of the other seven chemicals (mirex, hexachlorobenzene (HCB), nitrobenzene, ethyl formate, ethyl acetate, 2,4,5-trichlorophenol, and 2,4-dichlorophenol). Four fish out of the eight were reexposed with selected chemicals near the end of each 2- to 4-day experiment to see if changes in uptake efficiency had occurred for a given chemical over the duration of the experiment. Uptake efficiency values for the four reex-

[1] Glencoe Mills, Glencoe, Minn. 55336.

[2] Ayerst Laboratories, New York, N.Y.

TABLE 1

PHYSICAL–CHEMICAL INFORMATION ON EACH OF THE FOURTEEN $^{14}C$-TAGGED ORGANIC CHEMICALS USED TO DETERMINE THE EFFECTS OF LOG *P* ON THE TRANSPORT OF ORGANICS ACROSS TROUT GILLS

| Compound | Molecular weight | Specific activity (mCi/mmol) | Log *P* | Ref.[a] | Aqueous solubility (M) | Ref. | Exposure concentration | | 96-hr LC50 (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | M | μg/l | | |
| Ethyl formate | 74.08 | 5.25 | 0.23 | 1 | $1.19 \times 10^{0}$ | 1 | $3.7 \times 10^{-8}$ | 2.75 ± 0.11 (18)[b] | $2.3 \times 10^{5}$ | 17 |
| Ethyl acetate | 88.10 | 5.16 | 0.73 | 1, 2 | $9.1 \times 10^{-1}$ | 1 | $3.6 \times 10^{-8}$ | 3.21 ± 0.17 (22) | $2.3 \times 10^{5}$ | 17 |
| *n*-Butanol | 74.10 | 10.00 | 0.85 | 1, 2 | $1.062 \times 10^{0}$ | 1 | $2.5 \times 10^{-8}$ | 1.85 ± 0.04 (18)[c] | $1.7 \times 10^{6}$ | 18 |
| | | | | | | | $2.4 \times 10^{-8}$ | 1.80 ± 0.03 (18) | | |
| | | | | | | | $3.2 \times 10^{-8}$ | 2.37 ± 0.13 (18) | | |
| Nitrobenzene | 123.1 | 6.48 | 1.85 | 1, 2 | $1.4 \times 10^{-2}$ | 9 | $2.3 \times 10^{-8}$ | 2.81 ± 0.15 (20) | $4.3 \times 10^{4}$ | 19 |
| | | | | | $1.7 \times 10^{-2}$ | 1 | | | | |
| | | | | | $1.7 \times 10^{-2}$ | 13 | | | | |
| | | | | | $3.11 \times 10^{-2}$ | 6 | | | | |
| *p*-Cresol | 108.14 | 13.11 | 2.00 | 2 | $1.7 \times 10^{-2}$ | 4 | $3.5 \times 10^{-8}$ | 3.82 ± 0.23 (9) | $7.9 \times 10^{3}$ | 20 |
| Chlorobenzene | 112.6 | 28.2 | 2.84 | 2 | $4.3 \times 10^{-3}$ | 1 | $3.9 \times 10^{-9}$ | 0.443 ± 0.03 (15)[c] | $4.7 \times 10^{3}$ | 21 |
| | | | | | $4.0 \times 10^{-3}$ | 7 | $2.3 \times 10^{-9}$ | 0.263 ± 0.04 (38) | | |
| | | | | | $4.35 \times 10^{-3}$ | 4 | | | | |
| 2,4-Dichlorophenol | 163.0 | 4.43 | 2.93 | 2 | — | — | $5.5 \times 10^{-8}$ | 9.01 ± 0.32 (24) | $8.3 \times 10^{3}$ | 22 |
| Decanol | 158.29 | 16.5 | 3.09 | 2 | $2 \times 10^{-4}$ | 8 | $3.2 \times 10^{-8}$ | 5.07 ± 0.17 (36)[c] | $2.4 \times 10^{3}$ | 18 |
| | | | | | | | $1.9 \times 10^{-8}$ | 2.96 ± 0.09 (18) | | |
| Pentachlorophenol | 266.4 | 26.88 | 3.32 | 3 | $5.3 \times 10^{-5}$ | 9, 14 | $4.1 \times 10^{-10}$ | 0.108 ± 0.01 (18)[c] | $5.0 \times 10^{1}$ | 22 |
| | | | | | $7.5 \times 10^{-5}$ | 10 | $2.4 \times 10^{-10}$ | 0.064 ± 0.01 (12) | | |
| 2,4,5-Trichlorophenol | 197.5 | 6.79 | 3.70 | 2 | — | — | $2.3 \times 10^{-8}$ | 4.58 ± 0.41 (32) | $9.0 \times 10^{2}$ (24 hr) | 23 |
| Dodecanol | 186.3 | 10.31 | 5.10 | 2 | $1.0 \times 10^{-5}$ | 8 | $9.3 \times 10^{-9}$ | 1.73 ± 0.14 (38) | $1.0 \times 10^{3}$ | 18 |
| 2,5,2′,5′-Tetrachlorobiphenyl | 291.99 | 9.87 | 6.00 | 4 | $2.05 \times 10^{-8}$ | 11 | $9.7 \times 10^{-10}$ | 0.283 ± 0.087 (18)[c] | $7.7 \times 10^{0}$ | 24 |
| | | | | | $8.9 \times 10^{-8}$ | 4 | $3.7 \times 10^{-10}$ | 0.109 ± 0.028 (22) | | |
| | | | | | $9.08 \times 10^{-8}$ | 12 | | | | |
| Hexachlorobenzene | 284.8 | 14.07 | 6.18 | 5 | $1.9 \times 10^{-8}$ | 16 | $4.5 \times 10^{-11}$ | 0.0128 ± 0.0019 (16) | >Solubility | 25 |
| Mirex | 545.6 | 5.64 | 7.50 | 2 | $1.3 \times 10^{-10}$ | 15 | $5.2 \times 10^{-11}$ | 0.0286 ± 0.0065 (16) | $7.2 \times 10^{5}$[d] | 26 |

[a] References: (1) Hansch *et al.* (1968); (2) Hansch and Leo (1979); (3) Kaiser and Valdmanis (1982), note, apparent log *P*; (4) Neely (1982); (5) Neely *et al.* (1974); (6) Wasik *et al.* (1981); (7) *Handbook of Chemistry and Physics*, 64th ed. (1983–1984); (8) Peterson (1981); (9) Kenaga and Goring (1980); (10) Schmidt-Bleek *et al.* (1982); (11) Weise and Griffin (1978); (12) Hague and Schmedding (1975); (13) Banerjee *et al.* (1980); (14) Verschueren (1977); (15) Smith *et al.* (1977); (16) Hashimoto *et al.* (1982); (17) Veith (personal communication); (18) Veith *et al.* (1983); (19) Buccafusco *et al.* (1981); (20) DeGraeve *et al.* (1980); (21) Dalich *et al.* (1982); (22) Phipps *et al.* (1981); (23) Hattula *et al.* (1981); (24) Nebeker *et al.* (1974); (25) Benoit *et al.* (1984); (26) Carter and Graves (1972/73).

[b] Number of water analyses made.

[c] Compounds are retested at slightly different levels on the same or sequential fish.

[d] Value is greater than aqueous solubility.

posed chemicals differed from the initial values by ±6 to 15%, with a mean of 11%. This value was well below the range seen between individual animals (Fig. 1) and essentially the same as the percentage variation noted between repeated measurements made during the initial exposure period. In earlier investigations involving continuous 48-hr exposures, trout uptake efficiency values for tetrachlorobiphenyl and endrin varied by 1 to 15 and 3 to 8%, respectively (McKim and Heath, 1983; McKim and Goeden, 1982). Measurements were also made on the amounts of certain chemicals eliminated back out across the gills at the end of the 1-hr flush periods between chemical dosing. This procedure provided the necessary information to allow us to adjust uptake efficiency determinations for gill elimination and to gain insight into those chemicals which were very readily eliminated across the gills.

*Water characteristics.* All radiolabeled chemicals (purity > 98%) used in these experiments were supplied by Pathfinder Laboratories.[3] Specific activities listed in Table 1 were confirmed by liquid scintillation counting (LSC) then gas chromatographic (GC) analysis of each $^{14}C$-labeled compound using a series of nonradioactive standards for each compound in question. Analyses were done on a Hewlett–Packard 5730A automatic GC with Model 3552A data system. Eleven of the fourteen compounds were detected by flame ionization, with a nitrogen carrier gas, and under isothermal programming at column temperatures between 95 to 210°C and retention times of 1 to 4 min. Ethyl formate, ethyl acetate, *n*-butanol, 2,4-dichlorophenol, decanol, and 2,4,5-trichlorophenol were chromatographed on a Tenax-GC 60/80 mesh 3-ft × 2-mm column after direct aqueous injection. Nitrobenzene, *p*-cresol, chlorobenzene, and dodecanol were injected in hexane onto a 6-ft × 2-mm column of 10% Carbowax 20 M on 80/100 mesh Gas Chrom Q. The tetrachlorobiphenyl isomer was run on a 2-m × 2-mm column of 1.5% OV-17 and 1.95% QF-1 on 80/100 mesh Gas Chrom Q. The remaining three compounds were run using $^{63}Ni$ electron capture detection. Hexachlorobenzene and pentachlorophenol (after derivatization of the phenol with diazomethane) were chromatographed using a 1.5% SP-2250 and 1.95% SP-2401 on 100/120 mesh Supelcoport at 160 and 165°C, respectively. The carrier gas was 5% methane:95% argon. Mirex was the only compound analyzed by temperature programming (80 to 280°C at 4°/min and a 36.75-min retention time), with a hydrogen carrier gas and on a DB-5 capillary column 15 × 0.25 m.

The $^{14}C$-labeled-chemicals in microliter amounts of solvent were placed in empty 19-liter carboys. Most of the solvent was removed by gently blowing filtered air into the carboy before distilled water was added to bring the stock solutions to volume. Except for these trace amounts of solvents no carriers were used in these experiments. Each stock solution was delivered by an FMI[4] chemical-metering pump into the toxicant-mixing cell (McKim and Goeden, 1982) where it was diluted with 11°C water from Lake Superior at a flow rate of 600 ml/min. The $^{14}C$-labeled chemicals in solution then flowed out of the mixing cell at 300 ml/min through two flow-calibrated capillary tubes into two respirometer–metabolism chambers.

Mirex, because of its low water solubility, was mixed in four 19-liter glass carboys containing lake water (two for each respirometer–metabolism chamber) at the predetermined test concentration. These carboys were then brought to a temperature of 11°C in a water bath and vigorously stirred for 12 to 14 hr prior to use. During the exposure period, an FMI pump delivering 250 ml/min was used to pump the premixed mirex solution directly into the respirometer–metabolism chambers.

Some of the $^{14}C$-labeled chemicals in inspired and expired water [mirex tetrachlorobiphenyl, pentachlorophenol, and hexachlorobenzene (HCB)] were analyzed for radioactivity after solvent extraction, the rest were counted directly. Mirex required the extraction of 970 ml of exposure water using a mixture of 25 ml hexane and 5 ml toluene. The water was extracted twice and 10-ml aliquots of each extract were combined and evaporated to 5 ml and counted in 15 ml of nonaqueous Ready Solv-NA[5] solution in a Beckman LS 8000[5] liquid scintillation counter. HCB, TCB, and PCP water samples (225 ml) were all extracted with 25 ml hexane. In addition, the PCP water samples were acidified (pH < 3.5) prior to extraction. HCB solvent extracts were further concentrated from 10 to 5 ml before counting. Quantities of 5 ml PCP, TCB, and concentrated HCB extracts were mixed with Ready Solv-NA before 60-min counts. Spiked water samples yielded >94% recovery for all solvent extractions. The remaining ten chemicals were counted directly after mixing 1 ml of exposure water with 19 ml of an aqueous solution (Ready Solv-MP)[5] in a scintillation vial.

*Statistical analysis.* A *t* test was used to analyze all of the respiratory physiology data (Winer, 1971); whereas regression analysis was applied to the uptake efficiency vs time data (Neter and Wasserman, 1974).

## RESULTS

### *Respiratory Function*

There were no significant effects ($p \geq 0.05$) of any of the 14 chemicals tested on respi-

[3] Pathfinder Laboratories, St. Louis, Mo. 63141.

[4] Fluid Metering, Inc., Oyster Bay, N.Y. 11771.

[5] Beckman Instruments, Skokie, Ill. 60076.

ratory function (ventilation rate, ventilation volume, oxygen uptake efficiency) over the 2-day exposure period as summarized in Table 2. Using the same respirometer–metabolism chamber the values in Table 2 also agreed well with previous control data on brook and rainbow trouts (McKim and Goeden, 1982; McKim and Heath, 1983).

*Uptake Efficiency and Gill Transport vs Log P*

The gill uptake efficiencies for 14 different organic chemicals were plotted against each chemical's log *P*, which ranged from 0.23 for ethyl formate to 7.5 for mirex (Fig. 1). All of the chemical concentrations were well below calculated and measured solubilities (Table 1). The uptake efficiencies for three chemical with log *P*'s < 1 (ethyl formate 0.23, ethyl acetate 0.73, and *n*-butanol 0.85) were very low and showed no variations in uptake efficiency with changing log *P* (Fig. 1). However, from *n*-butanol with a log *P* of 0.85 to chlorobenzene at a log *P* of 2.84 the uptake efficiencies were directly related to log *P*. Beyond chlorobenzene to hexachlorobenzene (log *P* 6.18) uptake efficiency leveled off and again was not correlated to log *P*. Finally, beyond hexachlorobenzene uptake efficiency seemed to be inversely related to log *P* as observed in Fig. 1 for results with hexachlorobenzene (log *P* 6.18) and mirex (log *P* 7.5). This observation was further supported by data on the uptake efficiency and gill transport (Fig. 1) of the pyrethroid pesticide fenvalerate [calculated log *P* of 7.2 (Hansch and Leo, 1979)] determined at this laboratory under the same test conditions as the present study (Bradbury, 1983).

Uptake efficiency was converted to a 24-hr gill transport or flux rate (Fig. 1) by normalizing uptake efficiency in Fig. 1 to the lowest water concentration tested (mirex, $5.2 \times 10^{-11}$ M). This calculation was accomplished by multiplying the uptake efficiency for each chemical by the daily mean respiratory volume for all eight fish by the mirex–water concentration. This assumed that the uptake rates of the chemicals were constant over a range of concentrations (Branson *et al.*, 1975; Suguira *et al.*, 1978; McKim and Goeden, 1982), and that all chemicals tested were below solubility and in solution.

*Uptake Efficiency vs Time*

The uptake efficiency measurements for those four chemicals with log *P* from 0.85 to 2.84 showed a consistent downward trend during the 4-hr exposure period (Fig. 2). Regression analysis showed the slopes for chlorobenzene and nitrobenzene were significantly different from zero ($p = 0.01$). In contrast, no consistent downward trends were observed in uptake efficiency for those chemicals with log $p > 2.85$, and regression analysis also showed no significant differences in their slopes from zero ($p = 0.01$).

TABLE 2

A COMPARISON OF RESPIRATORY FUNCTION IN RAINBOW TROUT PRIOR TO AND FOLLOWING EXPOSURE TO 14 DIFFERENT ORGANIC CHEMICALS

| | Control | Exposed |
|---|---|---|
| Ventilation rate (No./min) | 60.00 ± 8.47 (26)[a] | 63.47 ± 11.60 (88) |
| Ventilation volume (ml/min) | 117.60 ± 29.60 (25) | 117.36 ± 29.30 (92) |
| $O_2$ uptake efficiency (%) | 62.54 ± 6.65 (24) | 61.97 ± 9.36 (93) |

[a] Mean ± SD (*n*).

## DISCUSSION

The relationship between uptake efficiency of trout gills and log *P* observed in this study was not a linear relationship over the entire range of log *P* tested. Instead, these data are consistent with the sigmoid curve derived from the Yalkowsky and Morozowich (1980)

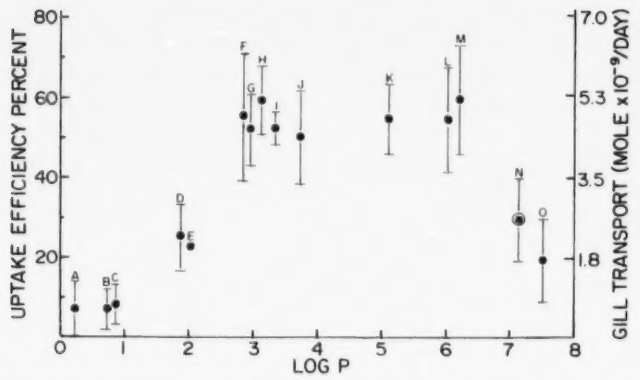


FIG. 1. The gill uptake efficiency and 24-hr gill transport of 14 selected organic chemicals as related to log *P*. Each point represents the mean of four trout (*p*-cresol = 1), while the bars around each point correspond to ±SD. Each chemical is identified by C, *n*-butanol; D, nitrobenzene; E, *p*-cresol; F, chlorobenzene; G, 2,4-dichlorophenol; H, decanol; I, pentachlorophenol; J, 2,4,5-trichlorophenol; K, dodecanol; L, 2,5,2',5'-tetrachlorobiphenyl; M, hexachlorobenzene; N, fenvalerate (Bradbury, 1983); O, mirex.

model, which differed considerably from the linear relationship of log *P* and bioconcentration factor (Veith *et al.*, 1979; Neely, 1979) or the linear relationship between gill uptake efficiency and log *P* calculated by Neely (1979). Hansch (1969) suggested an optimum value for log *P* existed above which the potential for uptake and biological activity decreased. Further, Hansch and Clayton (1973) reviewed and summarized a large volume of bioassay data that supported the original theory of nonlinearity between log *P* and biological response. They proposed that chemicals with very low log *P* values were so insoluble in fat they would not pass biological membranes, while those with very high log *P* became bound to the lipid membrane and could not pass into the blood or cellular fluids. Aquatic researchers have also suggested an optimum log *P* for the bioconcentration of chemicals in fish, noting a downward deflection of bioconcentration at the higher log *P* values (log *P* 6 to 8) (Zitko, 1974, 1977; Zitko and Hutzinger, 1976; Tulp and Hutzinger, 1978; Konemann and Van Leeuwen, 1980; Sugiura *et al.*, 1978). Our results on gill transport (flux) vs log *P* also provide evidence for a decreased uptake at the high log *P* values of 7.2 for fenvalerate (Bradbury, 1983) and 7.5 for mirex.

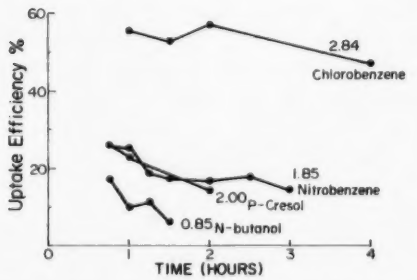


FIG. 2. The gill uptake efficiency of four organic chemicals with log *P* from 0.85 to 2.84 as related to exposure time. Each point represents the mean of four trout.

A physical model for diffusion was first presented by Zwolinski *et al.* (1949) and was further expanded by Stehle and Higuchi (1972), Ho *et al.* (1977), and Flynn and Yalkowsky (1972). This model described the rate of transport (flux) of a chemical across a biological membrane bounded by one or more aqueous compartments and is represented by Eq. (1):

$$F = \frac{\Delta C}{(R_m/PC) + R_{aq}} \quad (1)$$

$\Delta C$ = concentration differential across membrane and diffusion layers,
PC = membrane water partition coefficient,
$R_m$ = membrane resistance,
$R_{aq}$ = aqueous layer resistance
(see Flynn and Yalkowsky (1972) for derivation of this equation).

The model demonstrated that the diffusion of chemicals across biological membranes was controlled by both the lipid membrane and the nonmoving aqueous solvent layer (aqueous diffusion layer) at both the inside and outside surfaces of the membrane. Their studies indicated that low log $P$ chemicals with their high water solubility pass readily through the aqueous diffusion layers, but because of their low fat solubility are slowed down by the lipid membrane and have a low transport rate (Fig. 3). However, as chemicals increase in log $P$ they more readily pass the lipid membrane barrier and flux across the membrane is directly related to log $P$ (Fig. 3). As the log $P$ continues to increase there is eventually a transition point between membrane control and diffusion layer control. Finally, diffusion layer control takes over and there is no relationship between log $P$ and uptake (flux) across the membrane (Fig. 3). The membrane itself has no control at this point, because the high log $P$ chemicals are not hindered by the lipid membrane. At very high log $P$ values and high melting points, the water solubility of the chemical ultimately limits transport and the flux curve is deflected downward (Fig. 3).

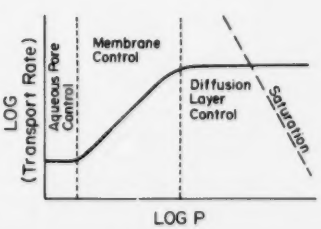


FIG. 3. Relationship of transport rate to partition coefficient when pore transport is not negligible (modified from Yalkowsky and Morozowich, 1980).

Yalkowsky and Morozowich (1980) used this same physical model, but added a second part [Eq. (2)] to account for the involvement of aqueous pores in the transport of low-molecular-weight (<100) highly polar compounds.

$$F = \frac{\Delta C}{R_m/PC + R_{aq}} + \frac{\Delta C}{R_p} \quad (2)$$

$R_p$ = total resistance of the aqueous pores
(see Yalkowsky and Morozowich (1980) for derivation of this equation)

Since only the molecular size of a molecule limits the movement through aqueous pores, there is no relationship to log $P$. Therefore, when pore transport is functioning the overall structure–activity curve is changed from the original hyperbolic shape (not including pore transport) to a sigmoid one (Fig. 3).

The empirical data on uptake efficiency of chemicals across the gills of fish observed in this study (Fig. 1) suggest a sigmoid relationship, consistent with the physical models of Flynn and Yalkowsky (1972) and Yalkowsky and Morozowich (1980). Furthermore, these data support and confirm the earlier contention that xenobiotics enter a fish by passive diffusion across the respiratory surface. One difference, however, between our data and those of Flynn and Yalkowsky (1972) was that fish were exposed to mirex and hexachlorobenzene at equal molar concentrations and below saturation, yet the flux of mirex was considerably lower than that of hexachlorobenzene. Fish were also exposed to fenvalerate (Bradbury, 1983) at concentrations well below solubility, yet gill flux rates were well below those for hexachlorobenzene in the present study. This result indicated the possibility that at these low test concentrations some physical–chemical characteristics other than solubility (i.e., molecular

weight, molecular volume, chemical self-association, etc.) was responsible for reduced membrane penetration at log $P$ above 7.

Data from the present study also demonstrate that the mechanisms controlling transport are nonspecific with respect to chemical structure, since 14 different chemicals were used from five major classes as compared to the homologous series used by Flynn and Yalkowsky (1972). The major controlling factors seem to be the membrane–water partition coefficient and the water solubility of the chemical as described by Yalkowsky and Morozowich (1980). Additional factors, such as molecular volumes, molecular weight, or self-association, may also play a part in gill transport at log $P > 7$.

Chemicals with log $P$ below 3 seemed to be eliminated back across the gills very rapidly (within 4 hr) whereas those above log $P$ 3 were not eliminated across the gills within the dosing period. Those chemicals above log $P$ 3 were so lipophilic that elimination back out across the gills is greatly reduced. In many cases, the higher log $P$ chemicals may require metabolism to increase polarity and water solubility, so that excretion via all routes can take place.

The bioconcentration factor is directly related to log $P$ within the range of 1 to 6 (Neely, 1979, Veith *et al.*, 1979). We also know from the gill transport data in the present that the uptake or transport rate is the same for those chemicals with log $P$ ranging from 2.84 to 6.18 and then decreases beyond log $P$ 6.18 (Fig. 3). Therefore, the slope of the log BCF vs log $P$ relationship between log $P$ 3 and 7.5 must be controlled in major part by excretion rates. Those chemicals with the higher log $P$ values have the slower elimination rates. This result was further supported by the strong inverse correlation shown between elimination rate and log $P$ developed in the review article by Spacie and Hamelink (1982). Veith *et al.* (1979) showed that the correlation between log BCF and log $P$ had an intercept of 0.7. Our data are consistent with this finding and indicate that the bioconcentration of chemicals from water at the gills cannot occur to any appreciable extent below log $P$ 1, because of the low flux rates associated with aqueous pore transport and the rapid attainment of steady state. The mirex data also suggested that chemicals with log $P$ above 8 might not be significantly accumulated across the gills of fish.

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**16. ABSTRACT**

An in vivo fish preparation was used that allowed a direct measure of the transport rates of 14 different organic chemicals across the gills of rainbow trout (Salmo gairdneri). The chemicals, all $^{14}C$ labeled, were selected from five classes, encompassing a range of octanol-water partition coefficient (log P) values, from 0.23 (ethyl formate) to 7.5 (mirex). The uptake efficiency (extraction efficiency) of each chemical was determined by monitoring the inspired and expired water of trout exposed to each chemical over an exposure period of 1 to 6 hr. The mean gill extraction efficiency for all chemicals tested varied from a low of 7% to a high of 60%, extracted in a single pass of the chemical across the gills. The extraction efficiency of chemicals with log P of 1 or less were low and showed no relationship to log P. These low extraction efficiencies seen at log P of 1 and below with molecular weights below 100 were indicative of aqueous pore transport. The mean extraction efficiency for chemicals with log P values of 1 to 3 seemed to vary directly with log P, to a maximum of slightly greater than 60%, suggesting that uptake was controlled by the lipid membrane. The mean extraction efficiency for chemicals with log P of 3 to 6 was independent of log P and remained at 60%, which suggested that gill uptake was controlled by aqueous diffusion rates rather than gill membrane permeability. The mean extraction efficiency with mirex (log P = 7.5) decreased to 20%. This behavior was consistent with previous physical models which predict that high log P and melting point (low water solubility) may substantially reduce the rate of accumulation in fish. These studies support the passive diffusion model for the uptake of organics at the gill-water interface.

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